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Effectiveness of Oral Killed Typhoid Vaccine*

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A controlled field trial of oral killed typhoid vaccine was carried out in Delhi, India in 1968–69. Altogether, 13 374 children below the age of 17 years were included in the study. Two comparable groups of children were given either placebo or vaccine. Each tablet of vaccine contained 100×10^9 *killed* Salmonella typhi, *and 3 tablets of vaccine or placebo were administered to each child. The vaccinated subjects were followed up from 10 June 1968 to 31 August 1969. The effectiveness of the vaccine was measured by comparing the incidence of typhoid fever (based only on bacteriologically positive cases) in the two groups. It was found that the difference in incidence of the disease in the two groups was not statistically significant. The oral killed typhoid vaccine in the dosage schedule used in the present trial was found not to be effective against the disease.*

Controlled field trials with parenteral vaccines against typhoid fever conducted in Yugoslavia (Yugoslav Typhoid Commission, 1962, 1964), British Guiana (now Guyana) (Typhoid Panel, UK Department of Technical Co-operation, 1964) Poland (Polish Typhoid Committee, 1966), and the USSR (Hejfec et al., 1966), under the sponsorship of World Health Organization (Cvjetanović & Uemura, 1965), have proved that these vaccines can provide a relatively high level of long-lasting immunity. An effective oral typhoid vaccine would however have certain advantages since repeated injections are not readily accepted by the people in developing countries.

The present study was undertaken to determine the effectiveness of an oral killed typhoid vaccine by a controlled field trial.

AREA AND POPULATION SELECTED FOR THE TRIAL

The study population consisted of children below the age of 17 years in a densely populated, low socio-economic community on the northern bank of the Jamuna River (Geeta Colony) in Delhi. The water supply was from shallow (16–20 ft; 5–6 m) tube wells with a separate hand-pump for each household. Dwellings were provided with bucket latrines and there were open drains for the removal of household waste. Each family cooked its own food. Fresh, unpasteurized milk obtained from local dairies was boiled before use. Numerous hawkers visited the colony to sell cut fruits and vegetables, ice cream and, other foods.

SIZE OF THE STUDY POPULATION

Since no data were available on the actual incidence of typhoid in Delhi, a rough estimate of 5.0–7.5 cases per 1 000 persons per annum was made after hospital records had been examined. It was observed that typhoid fever occurred more often in areas without a piped water supply.

On the basis of the estimated incidence of typhoid, a sample of 5 000 children per group was considered sufficient to demonstrate a statistically significant difference between the vaccinated and control groups if the effectiveness of the vaccine was at least 50%.

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The principles of controlled field trials were strictly observed. The placebo and vaccine tablets were labelled with code letters and the double-blind test method was used.

VACCINE

The placebo and vaccine tablets were given the code letters " A " and " B ", respectively, and were supplied by the World Health Organization.[1] Each tablet coded with the letter " B " contained 100×10^9 acetone-killed *Salmonella typhi*. The vaccine was declared safe for use by the manufacturer.[1] Tablets coded with the letter " A " contained lactose.

DESIGN OF THE STUDY

Population census

A house-to-house survey of the colony was made from 9 to 30 May 1968 by 20 research assistants. The members of each household were listed in family registers, together with data on age, sex, etc. When the census was complete, the names of all children aged 17 years and under were entered on a master sheet and numbered serially. Each family was given an identity card bearing the name and registered number of each child and was informed that the children were entitled to free medical treatment at the clinic which had been specially established in Geeta Colony. (The clinic had medical officers and a staff nurse to look after sick children from the colony. All the children in the colony, irrespective of the type of disease, attended the clinic.) The total number of children recorded in the census was 16 490.

Vaccination programme

The children were allocated to group A or group B according to whether the final digit in their serial number was odd or even. Each child was given 1 tablet daily on 3 alternate days or on 3 consecutive days; as a rule, tablets were administered 1½–2 hours after a meal.

Children who were suffering from either diarrhoea or fever at the time of vaccine administration were excluded from the list of the participants. Children who had received parenteral T.A.B. vaccine during the previous year were also excluded from the trial.

The vaccine was administered during the period from 10 June to 12 August 1968. Altogether, 13 374 children were treated with tablets A or B (Table 1).

Table 1. Distribution of children according to number of doses in the vaccine and control groups

No. of doses	Control group	Vaccine group	Total
1	359	347	706
2	176	184	360
3	6 248	6 060	12 308
total	6 783	6 591	13 374

All 3 doses could be administered to only 12 308 children, 1 066 children received only 1 or 2 doses for reasons such as age below 6 months, sickness, refusals, and movement away from Delhi (Table 2). A vaccination card was completed for each participant.

Surveillance

Each participant was followed up regularly from the first day of vaccination. Each research assistant was responsible for approximately 150–200 households, 60 households being visited every day by each assistant. In this way, each house was visited every fourth day. Details about the sickness of each child were entered in the family register during these visits.

All sick children, irrespective of the type of disease, were referred to the clinic where they were examined and treated. Patients with fever were taken personally to the clinic by the research assistants for diagnosis and treatment, and were followed up every day until they felt well. Those who had a high continuous fever lasting more than 3–4 days without any signs or symptoms of another disease were investigated for enteric infection. A 5-ml sample of venous blood was taken from the patient, 2 ml being injected in a sterile bottle containing sodium citrate for subsequent culture and 3 ml being injected into an empty sterile bottle for the Widal test and clot culture. These samples were sent to the Microbiology Department of Maulana Azad Medical College, New Delhi, for further bacteriological and serological examination.

Stool samples from all patients suspected of having typhoid fever were also sent to the Microbiology Department of Maulana Azad Medical College for culturing. During the first 3 months of the trial, the majority of the stool samples were collected in the first and second weeks of illness while the patients were already under treatment with chloromphenicol. Later, stool samples were collected from all patients

[1] The vaccine was produced by the Swiss Serum and Vaccine Institute, Berne, Switzerland.

Table 2. Distribution of children according to age and the number of vaccine doses in the vaccine and control groups

Age group (years)	Control group				Vaccinated group			
	1 dose	2 doses	3 doses	Total	1 dose	2 doses	3 doses	Total
<1	28	5	204	237	18	10	198	226
1–5	94	44	1 416	1 554	104	36	1 386	1526
6–10	105	53	1 996	2 154	106	59	1 942	2 107
11–15	95	56	2 024	2 175	97	60	1 989	2 146
16–17	37	18	608	663	22	19	545	586
total	359	176	6 248	6 783	347	184	6 060	6 591

before the chloromphenical treatment began, but no differences in isolation rates were noted.

Criteria for diagnosis

The following criteria were adopted for diagnosing typhoid fever:

(1) a blood culture positive for *S. typhi*;

(2) a stool culture positive for *S. typhi* in a clinically diagnosed case, with or without a positive blood culture.

These two criteria were considered when a case of typhoid was diagnosed for the purpose of evaluating the effectiveness of the vaccine.

Patients with a clinical diagnosis of typhoid and high or rising titres (1 : 400 and above) of O and H antigens, but in whom *S. typhi* could not be isolated, were considered as presumptive cases and are presented separately in the tables.

RESULTS

Over 70 000 episodes of sickness were recorded among the children in the trial during the period 10 June 1968 to 31 August 1969, and 537 patients were investigated bacteriologically and serologically for typhoid fever. Clinical follow-up of these patients revealed that only 283 had clinical features suggestive of this condition. Of these 283 subjects, 244 had taken one or more doses of vaccine or placebo.

Culture-positive patients

S. typhi were isolated from 110 of the subjects who participated in the trial; 96 of these isolations were made from blood cultures only while 11 were made from both blood and stool cultures. Positive stool cultures alone were found in only 3 clinically diagnosed cases, which also showed rising Widal titres for O and H antigens (Table 3).

Presumptive cases

An additional 82 patients had illness suggestive of typhoid fever and had either high (1 : 400 and above) or rising titres for O and H antigens on serological examination. In these patients, no organisms could be isolated in culture; they were considered as presumptive cases of typhoid and were analysed separa-

Table 3. Distribution of confirmed and presumptive cases of typhoid fever among the participants according to the number of vaccine doses

Diagnosis group	No. of doses	Control group	Vaccine group	Total
bacteriologically confirmed cases	1	1	2	3
	2	3	0	3
	3	60	44	104
	total	64	46	110
serologically and clinically diagnosed (presumptive) cases	1	1	2	3
	2	0	3	3
	3	36	40	76
	total	37	45	82

tely (Table 3). These presumptive cases were not included in the analysis of the effectiveness of the vaccine.

The number of culture-positive patients among the participants who were given only 1 or 2 doses of vaccine or placebo was small and these subjects were excluded from further statistical analysis.

Effectiveness of the vaccine

Evaluation of the vaccine, based on culture-positive patients with typhoid fever only (Table 4), revealed that 60 cases of typhoid fever occurred in the control group and 44 in the vaccine group during the period of study; that is, an incidence of 9.6 per thousand in the control and 7.3 per thousand in the vaccine group. The difference is not statistically significant ($P > 0.17$).

Table 4. Incidence of typhoid in the vaccinated and control groups from 10 June 1968 to 31 August 1969

Diagnosis group	Control group [a]		Vaccinated group [b]	
	No. of cases	Incidence per thousand	No. of cases	Incidence per thousand
Bacteriologically confirmed cases	60	9.6	44	7.3
serologically and clinically diagnosed (presumptive) cases	36	5.8	40	6.6
total	96	15.4	84	13.9

[a] 6 248 participants.
[b] 6 060 participants.

Similar analysis on presumptive cases of typhoid fever, i.e., clinically suggestive and serologically positive but culturally negative, revealed incidences of 5.8 and 6.6 per thousand, respectively, in control and vaccine groups. This difference is also not statistically significant. Combining the confirmed and presumptive cases, the respective incidences are 15.4 and 13.9 per thousand, and the difference is not significant (Table 4).

In order to determine whether any immunity occurred in the early stages of vaccination, the incidence of typhoid fever was compared in the control and vaccinated groups at 6-monthly intervals. The differences in the number of bacteriologically confirmed typhoid patients in the two groups at the end of first 6 months (22 in the control and 12 in the vaccine group) and at the end of the second 6 months (26 in the control and 14 in the vaccine group) are not significant ($P > 0.10$, $P > 0.05$, respectively; Table 5). A similar analysis of the total number of confirmed

Table 5. Incidence of typhoid fever in the control and vaccinated groups analysed at 6-monthly intervals. the basis for diagnosis being bacteriologically positive blood or stool samples

No. of days from vaccination to onset of fever	Control group [a]		Vaccinated group [b]	
	No. of cases	Incidence per thousand	No. of cases	Incidence per thousand
0–180	22	3.5	12	2.0
181–360	26	4.2	14	2.3
361–448	12	1.9	18	3.0

[a] 6 248 participants.
[b] 6 060 participants.

and presumptive cases (Table 6) also showed no significant difference in incidence in the vaccinated and control groups.

Detailed analyses based on age-specific incidence rates were made to determine whether there was any significant difference in the development of immunity at various ages (Table 7). It was observed that the incidence of typhoid fever in the 1–5 years and

Table 6. Incidence of total typhoid fever (confirmed and presumptive cases) in the control and vaccinated groups analysed at 6-monthly intervals, the basis for diagnosis being bacteriologically positive blood or stool samples (confirmed cases) and serologically positive patients (presumptive cases)

No. of days from vaccination to onset of fever	Control group [a]		Vaccinated group [b]	
	No. of cases	Incidence per thousand	No. of cases	Incidence per thousand
0–180	43	6.9	29	4.8
181–360	37	5.9	28	4.6
361–448	16	2.6	27	4.5

[a] 6 248 participants.
[b] 6 060 participants.

Table 7. Age-specific incidence of bacteriologically confirmed case of typhoid fever in the vaccine and control groups

Age group (years)	Control group			Vaccine group		
	No. vaccinated	No. of cases	Incidence per thousand	No. vaccinated	No. of cases	Incidence per thousand
< 1	204	1	4.9	198	0	0
1–5	1 416	16	11.3	1 386	11	7.9
6–10	1 996	25	12.5	1 942	17	8.8
11–15	2 024	14	6.9	1 989	14	7.0
16–17	608	4	6.6	545	2	3.7
total	6 248	60	9.6	6 060	44	7.3

6–10 years age groups was 11.3 and 12.5 per thousand respectively, in the control group and 7.9 and 8.8 per thousand in the vaccine group. These differences are not significant at the 5% level ($P>0.30$, $P>0.20$, respectively).

DISCUSSION

There was a high incidence of typhoid fever in the community under study. Cases of typhoid fever occurred all year round, but there was a significantly higher incidence during the period March–September (Table 8). The overall incidence of typhoid fever in participants in the trial (based on culture-positive cases only) after 448 days of the follow-up was 9.6 per thousand in the control group and 7.3 per thousand in the vaccine group. This difference is not statistically significant.

Analysis of the data at 6-monthly intervals showed that the incidence of typhoid fever in the first 6 months was 3.5 and 2.0 per thousand in the control and vaccine groups, respectively and, 4.2 and 2.3 per thousand in the second 6 months of the trial. Again, the differences are not significant. During the third period of 6 months, the incidence was 1.9 and 3.0 per thousand in the control and the vaccine groups, respectively.

The results of the trial indicate that oral killed vaccine is not effective against *S. typhi* infection in the dosage schedule employed.

No complications or deaths due to typhoid occurred in either of the groups, probably because the disease was detected in the very early stages and effectively treated. This was an ideal community in which to conduct a vaccination trial since the disease was highly endemic with an incidence rate

Table 8. Monthly distribution of bacteriologically confirmed typhoid cases in the vaccine and control groups

Period	Control group	Vaccine group	Total
1968			
10–30 June	0	0	0
1–31 July	2	3	5
1–31 Aug.	10	3	13
1–30 Sept.	1	3	4
1–31 Oct.	5	2	7
1–30 Nov.	2	0	2
1–31 Dec.	2	1	3
1969			
1–31 Jan.	0	0	0
1–28 Feb.	1	0	1
1–31 Mar.	10	2	12
1–30 Apr.	5	6	11
1–31 May	5	4	9
1–30 June	6	5	11
1–31 July	3	2	5
1–31 Aug.	8	13	21
total	60	44	104

based on culture-positive cases of 9.6 per thousand in children below the age of 17 years.

Although this study indicated that the oral killed vaccine was not particularly effective, a further trial is now being conducted in another community with a similar vaccine containing a greater number of bacteria per dose. The results of the second trial should soon be known.

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RÉSUMÉ

EFFICACITÉ D'UN VACCIN ANTITYPHOÏDIQUE TUÉ ADMINISTRÉ PAR VOIE BUCCALE

On a organisé un essai contrôlé à Delhi (Inde) afin d'évaluer le pouvoir protecteur d'un vaccin antityphoïdique tué administré par voie buccale. Au total, 13 374 enfants de moins de 17 ans y ont participé.

Trois doses d'un vaccin renfermant 100×10^9 *Salmonella typhi* par dose ont été données en 3 jours, par voie buccale, à 6060 enfants; 6248 autres (groupe témoin) ont reçu 3 doses de placebo; pour diverses raisons, 1066 enfants n'ont reçu qu'une ou deux doses de l'une ou l'autre préparation.

La vaccination, commencée en juin 1968, a pris fin en août 1968 et chaque participant a été observé régulièrement, tous les 4 jours, jusqu'au 31 août 1969. Les enfants fiévreux ont été examinés par un médecin; en cas de fièvre persistante (plus de 3-4 jours), on a pratiqué une hémoculture, un examen sérologique et une coproculture. Seuls les cas de fièvre typhoïde confirmés bactériologiquement ont été retenus pour l'analyse des résultats.

Des contrôles bactériologiques et sérologiques ont été effectués chez 537 enfants fiévreux dont 283 présentaient des symptômes évoquant la fièvre typhoïde. On a isolé *S. typhi* chez 110 malades, 96 fois par hémoculture seule, 11 fois par hémoculture et par coproculture, et 3 fois uniquement par coproculture. Sur ces 110 typhiques, 44 avaient reçu les 3 doses de vaccin et 60 les 3 doses de placebo; l'incidence dans le 1er groupe était de 7,3 pour 1000 et dans le second de 9,6 pour 1000, la différence étant statistiquement non significative ($P > 0,17$).

Afin de s'assurer de l'existence éventuelle d'une immunité postvaccinale précoce, on a comparé l'incidence dans les deux groupes à 6 mois d'intervalle. On n'a relevé de différences significatives ni au 6e mois ($P > 0,10$) ni au 12e mois suivant la vaccination ($P > 0,05$). L'incidence relative aux groupes d'âge 1-5 ans et 6-10 ans a atteint 11,3 et 12,5 pour 1000 dans le groupe témoin et 7,9 et 8,8 pour 1000 dans le groupe vacciné, les différences n'étant pas significatives ($P > 0,30$ et $P > 0,20$ respectivement).

Ces résultats indiquent que le vaccin antityphoïdique tué administré par voie buccale aux doses utilisées dans le présent essai ne confère aucune protection contre la fièvre typhoïde.

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